Long-term effects of early parasite exposure on song duration and singing strategy in great tits

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Abstract: Song is a sexually selected trait in many bird species and has been suggested to function as a signal of a male's health and parasite resistance. Here we present an experimental field study on the long-term effects of parasite exposure early in life on adult bird song. We exposed nestling great tits (Parus major) to ectoparasitic hen fleas (Ceratophyllus gallinae) and on their recruitment into the local breeding population assessed the response to a playback of a challenging male in their breeding territory. We show, to our knowledge for the first time in a wild bird population, that parasite exposure early in life affects bird song: song duration of males that were exposed to parasites early in life was reduced by 32% compared with males that grew up in a flea-free environment. Early parasite exposure also significantly reduced the degree of song overlap with the playback, which has been shown to correlate with social status. There was no effect of early parasite exposure on the number of different song types sung or on the latency until the males started the vocal response to the playback. These results suggest that mates or rivals can use song duration and song overlap as proxies for an individual's exposure to parasites early in life. It thereby highlights both the importance of parasites in maintaining honesty of sexually selected traits and the costs of parasitism in terms of reduced attractiveness and competitiveness.

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Longterm effects of parasite exposure early in life on song duration and singing strategy – a case study in great tits

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ABSTRACT

Song is a sexually selected trait in many bird species, and has been suggested to function as a signal for a male's health and parasite resistance. Here we present an experimental field study on the long-term effects of parasite exposure early in life on adult bird song. We exposed nestling great tits (*Parus major*) to ectoparasitic hen fleas (*Ceratophyllus gallinae*), and upon their recruitment into the local breeding population assessed the response to a playback of a challenging male in their breeding territory.

The duration of the singing response of breeding males that had undergone post-natal exposure to parasites was reduced by 32% compared to males that grew up in a parasite-free environment, and also significantly reduced the degree of song overlap with the playback, which is thought to correlate with social status. There was no effect of early parasite exposure on the number of different song types sung within 20 minutes, or on the latency until the males started the vocal response to the playback. The results thus suggest, to our knowledge for the first time in an experimental field study, that mates or rivals can use song duration and song overlap as proxies for an individual's exposure to parasites early in life.

1. INTRODUCTION

The song of male birds often has a dual signalling function in both female attraction and repulsion of competing males (Darwin 1871; Catchpole 1982; Searcy and Andersson 1986; Catchpole and Slater 1995). Singing is energetically costly for males (Brackenbury 1979; Lambrechts and Dhondt 1988; Vehrencamp et al. 1989; Grafen 1990; Eberhardt 1994), implying that higher quality males can afford to display more costly song types, song structures or song rates. Numerous studies have shown that a male's present condition influences song rate and song repertoire (e.g., Buchanan et al. 1999; Berg et al. 2005). More recently, it has been suggested that a male's song might also be affected by environmental conditions experienced early in life. Indeed, several studies (Nowicki et al. 2002; Buchanan et al. 2003) have shown that nutritional stress during early development can affect song development (i.e., the nutritional stress hypothesis, Nowicki et al. (1998)). This hypothesis has been expanded to other stress factors, for example early parasite exposure or increased corticosterone levels, that might have long-lasting effects on song characteristics in adulthood (i.e., the developmental stress hypothesis, Spencer et al. (2003); Buchanan et al. (2004); Nowicki & Searcy, (2004); but see Gil (2006)).

Song has thereby the potential to function as a multiple signal of both a male's past growth and development (Buchanan et al. 2003; Spencer et al. 2003; Spencer et al. 2005a), and / or of his current condition (Buchanan et al. 1999). By assessing song rates and song structures during mate choice, females might thus not only gain honest information about a male's current status, but potentially also on past environmental conditions experienced by the male early in life (Catchpole 1996; Buchanan 2000; Buchanan and Catchpole 2000; Doutrelant et al. 2000).

Honest information about male quality is not only relevant for female mate choice, but also for male-male competition. Males often eavesdrop on the neighbour's song to get honest information on the motivation, condition or health status of potential contrahents (Peake et al. 2005). Two important aspects of intra-sexual vocal signalling are song matching, where an individual repeats the song pattern of the competitor (Krebs et al. 1981; McGregor et al. 1992; Nielsen and Vehrencamp 1995; Beecher et al. 2000; Vehrencamp 2001), and song overlapping, where an individual, depending on its relative dominance status, times its song relative to the opponent's song with various degrees of overlap (Dabelsteen et al. 1997).

To date, studies testing the effects of early environmental conditions on song development have been performed mainly under lab or aviary conditions (Buchanan et al. 2003; Spencer et al. 2004), while in the wild evidence for effects of stress experienced early in life on song development remains scarce. Here we investigated if early developmental stress, resulting from exposure to a common ectoparasite during the nestling period, can affect song traits and song overlap of males under natural conditions. We thereto experimentally infested nestling great tits (*Parus major*) after hatching with hen fleas (*Ceratophyllus gallinae*) and recorded their song after recruitment into the local breeding population the following years. Hen fleas are common ectoparasites of great tits. They live in the nest material of their hosts and suck blood from the nestlings (Tripet and Richner 1997b). If early developmental conditions have long-term consequences for adult song we predict a shorter duration of singing and / or a smaller number of different song types sung within the recording period in males that were exposed to hen fleas early in life. Furthermore, we predict that males raised in a parasite-free nest are more competitive and thus show a higher degree of song overlap with a competing male.

2. MATERIAL AND METHODS

a) Manipulation of early parasite exposure

The study was performed in a population of great tits breeding in nest-boxes in a forest close to Bern, Switzerland („Forst“, 46°54’N 7°17’E / 46°57’N 7°21’E). During the breeding seasons of 1998 – 2004 we manipulated the load of hen fleas in the nests. First, we microwaved the nesting material of all nests two days after hatching of the nestlings. This procedure kills all nest-based ectoparasites naturally present in the nests (Richner et al. 1993; Tripet and Richner 1997a). Thereafter, half of the nests were experimentally infested with 40 female and 20 male fleas originating from old nests collected within the same forest before the breeding season, while the other nests remained flea-free. Nests were randomly assigned to one of the two treatment groups. All nestlings were ringed with individually numbered aluminium rings when 9 days old. Fifteen days post-hatching, towards the end of the nestling stage, the nestlings’ body mass and metatarsus length were measured.

Twenty-two males (13 from experimentally infested nests and 9 from nests without parasites) were recaptured as breeders in 2005 in the local population. All recruits originated from different broods (i.e., they were not siblings). One of these males was born and manipulated in 1998 (no fleas), three males were born and manipulated in 2002 (two with fleas and one without fleas), and the rest of the males were born and manipulated in 2004. In summary, our sample consisted of 21 males, 17 of them were 1st year breeders and four of them older. Of the four older birds, two had been experimentally infested as nestling and two came from uninfested controls.

b) Recording and analysis of song

113 To create the song stimuli, we recorded the songs of five males from outside of our
114 study area ("Birchiwald", Bremgarten b. Bern, 46°58'N 7°25'E, Switzerland, distance
115 to study area ~10 km) from a distance of approximately 10 – 15 meters during 45
116 minutes each. The songs were recorded with a condenser shot gun microphone (Georg
117 Neumann, KMR 82 i) via a preamplifier (SHURE, FP23) to the program Sound Stu-
118 dio 2.1 (Kwok 2003) on a laptop. We used these digitised songs to create five differ-
119 ent song stimuli of 20 minutes duration in the programs Sound Studio 2.1 (Kwok
120 2003) and Raven 1.2.1 (Charif et al. 2004) (see details below).

121 The song of great tits has a typical structure (Figure 1): a combination of 1 – 5
122 notes (Krebs 1976; McGregor and Krebs 1982b) is called a phrase (about 0.2 – 0.5
123 sec; Figure 1). Phrases are usually repeated and form a strophe (McGregor and Krebs
124 1982b; Lambrechts and Dhondt 1986). Strophes are typically separated by an inter-
125 strophe pause before they are repeated again. Repeated strophes based on the same
126 kind of phrase are called a song type (sometimes also called song unit, Lambrechts
127 and Dhondt (1988)). A male great tit has a song repertoire of 1 to 9 distinct song types
128 (McGregor et al. 1981; McGregor and Krebs 1982a; Otter et al. 1999). Females are
129 attracted to males with larger song repertoires (Baker et al. 1986). Generally, great tits
130 sing strophes of the same song type for several minutes before switching to another
131 song type (Krebs 1976).

132 Some song types are much more common than others. The most common song
133 type in our study population (Figure 1) also occurred in all recordings of the five birds
134 used to create the different song stimuli. Single phrases of these most common song
135 type were extracted and multiplicated six times using Sound Studio 2.1 (Kwok 2003)
136 to create a stimulus strophe of 4 seconds followed by 3 seconds of silence (i.e., the
137 interstrophe pause, Figure 1). The duration of strophe and pause was based on a pre-

liminary study and together represents an average male's song unit in the study population (L.B. unpublished data). The song unit (i.e., the strophe and pause) was then repeated to create 1-minute stimulus sequences. Each stimulus contained the song of one male only. This approach allowed us to standardize the stimulus structure, while still covering a range of natural variation in song between different males. The amplitude of the five song stimuli were very similar, that is 83 ± 2 dB, measured at a distance of 1m in front of the loudspeaker using a sound pressure level meter (Voltcraft Plus, SL-300).

We recorded the males' response to a playback during the breeding season 2005. All recordings were performed between 6am – 1pm on the day the clutch contained the fourth egg. We played back a non-interactive 20-minute song stimulus in the male's breeding territory using an iPod mini with the full uncompressed WAVE/AIFF format setting and a loudspeaker (YAMAHA, AA5), which was placed one meter away from the nest box on the ground. The amplitude of the stimulus songs was standardized by using fixed settings on both the playback machine and the speaker.

For the song recordings of the target males, we first played a 5-minute song stimulus sequence (as described above) in each male's territory, followed by 30 seconds of silence. Then, we played six times a 2-minute song stimulus followed by a 30 seconds of silence to make up for a total of 20 minutes of playback in each male's territory. Thus each target male was exposed to the song of one male used as a stimulus selected at random from the five available stimuli (systematic randomization to assure that all stimuli were used equally often). All stimuli were played back to males originating from both parasite-infested and parasite-free nests respectively (stimulus 1: without fleas $n = 3$, with fleas $n = 2$; stimulus 2: without fleas $n = 1$, with fleas $n =$

163 3; stimulus 3: without fleas n = 2, with fleas n = 2; stimulus 4: without fleas n = 1,
164 with fleas n = 3; stimulus 5: without fleas n = 2, with fleas n = 3), and stimulus num-
165 ber (1-5) was included as a random effect in the statistical analyses to control for vari-
166 ation in the males' response due to variation among stimuli.

167 The song of the focus males was recorded during the entire 20-minute trial pe-
168 riod using a condenser shot gun microphone (Georg Neumann, KMR 82 i) via a pre-
169 amplifier (SHURE, FP23) to the program Sound Studio 2.1 (Kwok 2003) on a laptop.
170 The spectrograms were analysed blindly with respect to the parasite treatment of the
171 males (at the time they were nestlings) using the program Raven 1.2.1 (Charif et al.
172 2004). We measured the summed duration of all song units (see Figure 1 and above
173 for the definition of a song unit, i.e. the combined duration of strophe plus interstro-
174 phe pause) during the 20 minute recording (thereafter termed song duration), the
175 number of different song types sung within these 20 minutes, the latency until the
176 focus male responded to the playback, the summed duration of all strophe lengths
177 during the 20 minutes (termed after stroph length), and the summed duration of all
178 interstrophe pauses during the 20 minutes (termed after interstrophe pause length).
179 We also assessed the number of times that a target male sang on top of the stimulus in
180 order to calculate song overlap as follows: only cases where the target bird responded
181 *after* the stimulus strophe (for definition of song strophe see Figure 1) had started
182 were included, but all cases where the target male started *before* the stimulus strophe
183 were discarded from this analysis since in this cases song overlap may have arisen
184 just by chance. Percentage of song overlap was then calculated as the number of over-
185 lapping strophes / number of total strophes *100.

186

187 ***c) Clutch size and breeder morphology***

188 Clutch size was recorded at the start of incubation. Breeding parents were captured 12
189 days after hatching of their brood using a door trap inside the nest box, and their
190 metatarsus length and body mass were measured. Their body condition was calculated
191 as the residuals of a regression of body mass on tarsus length. The change in body
192 mass (Repeated measures ANOVA: $F_{1, 20} = 1.957$, $P = 0.177$) and metatarsus length
193 (Repeated measures ANOVA: $F_{1, 20} = 2.870$, $P = 0.106$) between fledging and adult-
194 hood did not differ significantly between males that had been parasite-exposed or
195 unexposed as nestlings.
196
197 ***d) Statistical analyses***
198 We used general linear models to analyse the effect of early flea exposure on the song
199 duration, the number of different song types sung within these 20 minutes, the latency
200 until the male started their vocal response to the playback, the strophe length, the in-
201 terstrophe pause length, and the percentage of song overlap. Song stimulus type (1 –
202 5) was included as a random factor in all statistical analyses to control for differences
203 in a male's vocal response due to variation among playback stimuli. Residuals of the
204 models were tested for normality using Shapiro-Wilk tests and homoscedasticity us-
205 ing Bartlett tests to ensure that the assumptions for parametric testing were fulfilled.
206 All tests were two-tailed with a significance level of 0.05. Means ± 1 SE are presented
207 in the results and the figures. Statistical analyses were performed in the programs R
208 2.6 (R Development Core Team 2004) and JMP IN 5.1 (Sall and Lehmann 1996).
209
210 **3. RESULTS**
211 ***a) Effects of experimental flea exposure on song***

212 Males raised in a parasite-infested nest responded vocally for a significantly shorter
213 period (364 ± 55 sec, n = 13) during the 20 minute playback trials than males brought
214 up in a parasite-free nest (536 ± 39 sec, n = 9; $F_{1, 16}$ = 5.396, P = 0.034). This longer
215 song duration of parasite-free birds was due to a tendency for longer strophe length (F
216 $_{1, 16}$ = 4.179, P = 0.057) as well as longer interstrophe pauses ($F_{1, 16}$ = 8.595, P =
217 0.009). Males raised in a parasite-free nest also overlapped their song significantly
218 more with the playback (62.9% ± 4.4) than males from an infested nest (48.3% ± 2.4;
219 $F_{1, 16}$ = 10.209, P = 0.006). This effect became even more significant ($F_{1, 15}$ = 21.972,
220 P < 0.001) when including song duration ($F_{1, 15}$ = 8.634, P = 0.010) as a covariate into
221 the analysis of song overlap, showing that early flea exposure influenced song overlap
222 and song duration independently (Figure 2).
223 Early flea exposure did not significantly influence the number of different
224 song types sung within these 20 minutes (with fleas: 3.3 ± 0.6, without fleas: 3.0 ±
225 0.4; $F_{1, 16}$ = 0.171, P = 0.684), or the latency until the males started their vocal re-
226 sponse to the playback (with fleas: 170.1 ± 63.9 sec, without fleas: 148.2 ± 98.3 sec;
227 $F_{1, 16}$ = 0.038, P = 0.847).
228
229 ***b) Correlates of song***
230 Song duration during the playback trials was not significantly influenced by a male's
231 body condition as an adult ($F_{1, 16}$ = 0.137, P = 0.716) or as a nestling ($F_{1, 16}$ = 0.004, P
232 = 0.948), and it was independent of male age ($F_{1, 16}$ = 0.003, P = 0.954), or the date
233 ($F_{1, 16}$ = 0.408, P = 0.532) or time of day of the song recording ($F_{1, 16}$ = 0.475, P =
234 0.501).
235 Similarly, song overlap was not significantly influenced by a male's nestling
236 body condition ($F_{1, 16}$ = 0.094, P = 0.763), male age ($F_{1, 16}$ = 0.848, P = 0.371), or the

237 time of day of song recording ($F_{1, 16}$ = 0.147, P = 0.706). We found a tendency for
238 males in better adult body condition to overlap their song more with the playback ($F_{1,}$
239 $_{16}$ = 3.846, P = 0.068), and also a decrease of song overlap with the ongoing breeding
240 season ($F_{1, 16}$ = 4.275, P = 0.055). The effect of the flea treatment on song overlap
241 remained significant ($F_{1, 14}$ = 8.274, P = 0.012) when including adult condition and
242 date of song recording as covariates into the statistical model.

243 Females laid larger clutches when mated to a male with a longer song duration
244 ($F_{1, 16}$ = 5.138, P = 0.038, Figure 3). No other aspect of song was significantly corre-
245 lated with clutch size (all P > 0.160) or male adult condition (P = 0.343). There was
246 no significant correlation between female and male condition ($F_{1, 19}$ = 0.387, P =
247 0.541), indicating that there was no assortative mating for body condition.

248

249 **4. DISCUSSION**

250 Several studies have shown that the brain structures associated with song learning are
251 costly to produce and thus vulnerable to stress (e.g., Catchpole 1996; Nowicki et al.
252 1998; but see Gil et al. 2006). In swamp sparrows (*Melospiza georgina*), for example,
253 nutritional limitation during growth had a negative effect on the development of the
254 song nuclei in the brain (Nowicki et al. 2002). Similarly, nestling development was a
255 predictor of adult song repertoire size in great reed warblers (Nowicki et al. 2000).
256 Furthermore, male european starlings (*Sturnus vulgaris*) that experienced an unpre-
257 dictable food supply early in life produced a smaller repertoire of song phrases than
258 males with a constant food supply (Spencer et al. 2004).

259 Beside these well documented effects of nutritional stress on song develop-
260 ment, a recent study by Spencer et al. (2005a) demonstrated that an infection with
261 blood parasites early in life can similarly affect song development. Captive male ca-

naries (*Serinus canaria*) that were infected with malaria parasites (*Plasmodium relictum*) during development produced simpler songs (number of different syllables) as adults and had a significantly smaller volume of the HVC nucleus (i.e., the "high vocal centre" in the brain) compared to uninfected males. Our study demonstrates that similar effects of early parasite exposure on song development occur in the wild as well: Male great tits that had been exposed to ectoparasitic hen fleas early in life sang for a shorter period during the playback trials than unexposed males. This is in agreement with the hypothesis that song development is costly (Eberhardt 1994; Gaunt et al. 1996), and it indicates that parasites might mediate the trade-off between investing limited resources in the development of song-related brain structures and parasite defence respectively. It thereby highlights the importance of parasites in ensuring honesty of sexually selected traits (Hamilton and Zuk 1982).

Interestingly, the effect of early parasite exposure on song duration were independent of adult or nestling condition. Furthermore, the number of different song types sung within the recording period and the latency until the male started their vocal response to the playback were not affected by the infestation experiment. This is surprising because Spencer et al. (2004, 2005a) have found that nutritional stress affects song repertoire size in passerines. It suggests that food restriction and parasite exposure affect song development in different ways, and it shows that song does not only contain multiple information about a male's condition over different time-scales (see also Scheuber et al. 2003a, 2003b), but also specific information about the specific type of stressor a male was exposed to early in life.

We found a positive correlation between a male's song duration and his partner's clutch size, which indicates that the duration of singing is a sexually selected trait in our study population (see also Houtman 1992; Collins et al. 1994; Gil and Gahr 2002;

287 Garamszegi and Møller 2004; Spencer et al. 2005b). This effect is unlikely due to
288 assortative mating because male and female condition were not significantly correlat-
289 ed. It thus demonstrates the benefits of a high song output for a male's fitness and
290 reveals the cost of parasitism for males in terms of reduced attractiveness.

291 Beside the effects of early ectoparasite exposure on the duration of singing,
292 which might be especially important in inter-sexual selection (see above), we also
293 found that parasite-exposed males overlapped the song of a challenger (i.e., the play-
294 back song stimulus) less than unexposed males. Several studies have demonstrated
295 that song overlapping is important in male-male competition as a signal of aggres-
296 siveness (Todt 1981; Brindley 1991; McGregor et al. 1992; Dabelsteen et al. 1996;
297 Dabelsteen et al. 1997; Naguib et al. 1999; Langemann et al. 2000; Mennill and
298 Ratcliffe 2004b), and dominance (Hyman et al. 2004; Mennill and Ratcliffe 2004b,
299 2004a). Males that overlap their opponent's song in male-male competition may
300 therefore be more likely to find a mating partner or to gain access to higher quality
301 territories, and might thereby achieve a higher reproductive success. In support of this
302 hypothesis, Kunc et al. (2006) found that the subsequent pairing success of male
303 nightingales (*Luscinia megarhynchos*) was related to the frequency of song overlap in
304 male-male interactions during the time of mate attraction. Also, in a dual-speaker
305 choice experiment female little blue penguins (*Eudyptula minor*) preferred the song of
306 males with a higher degree of song overlap (Miyazaki and Waas 2002). Furthermore,
307 in an experiment with alternating and overlapping playback, corn bunting males (*Mil-*
308 *iaria calandra*) approached the loudspeaker more quickly and spent more time close
309 to the loudspeaker when the playback was alternated rather than when it was overlap-
310 ping with their own song (Osiejuk et al. 2007), indicating that competitors are less
311 likely to challenge overlapping singers (Todt and Naguib 2000; Osiejuk et al. 2004).

312 Together, these studies suggests that parasite exposure early in life does not only af-
313 fect a male's attractiveness, but also his territory defence behaviour and competitive-
314 ness in intra-sexual encounters by altering its singing strategy.

315 To summarise, we experimentally show, to our knowledge for the first time in
316 a wild bird population, that parasite exposure early in life affects song duration and
317 the timing of singing of adult great tits. It demonstrates that song development is cost-
318 ly and highlights both the importance of parasites in maintaining honesty of sexually
319 selected traits and the costs of parasitism in terms of reduced attractiveness and com-
320 petitiveness.

321

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331

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490 **FIGURE LEGENDS**

491 Figure 1 Spectrogram of a typical great tit song.

492

493

494 Figure 2 Relationship between song duration and song overlap of adult males raised
495 in a flea-infested (n = 13; black) or a parasite-free nest (n = 9; white). The triangles
496 indicate old males (year of birth shown), the points indicate one-year old males (born
497 in 2004). Song overlap (% number of strophes overlapping with playback) and song
498 duration were recorded during a 20-minute period in response to a playback.

499

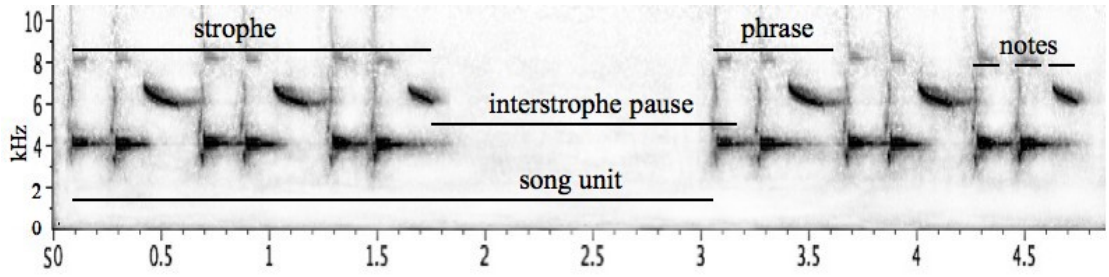
500

501 Figure 3 Relationship between song duration and clutch size. Black signs indicate
502 males infested with fleas early in life (n = 13), white signs represent uninfested males
503 (n = 9). The triangles indicate old males (year of birth shown), the points indicate one-
504 year old males (born in 2004). Song duration was recorded during a 20-minute period
505 in response to a playback.

506

507 **FIGURES**

508 Figure 1



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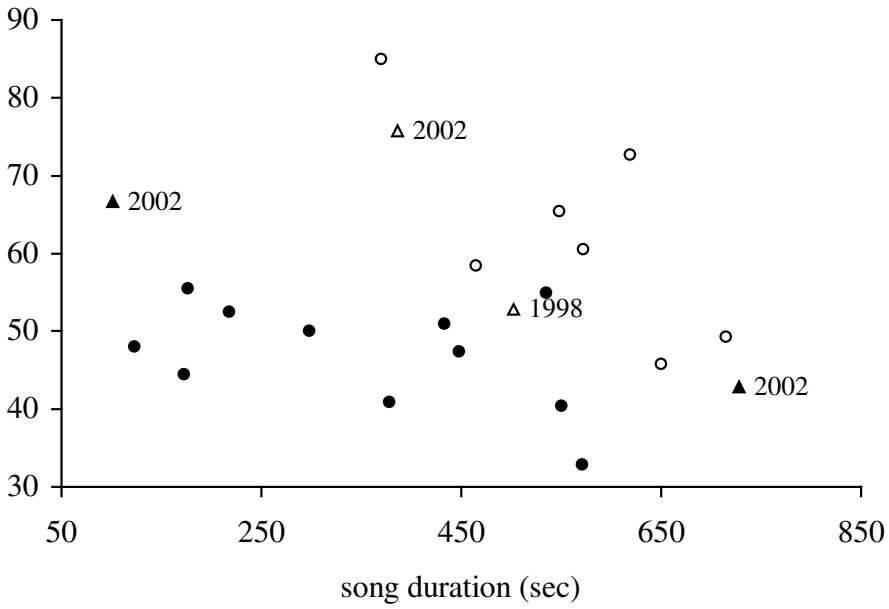
511 Figure 2



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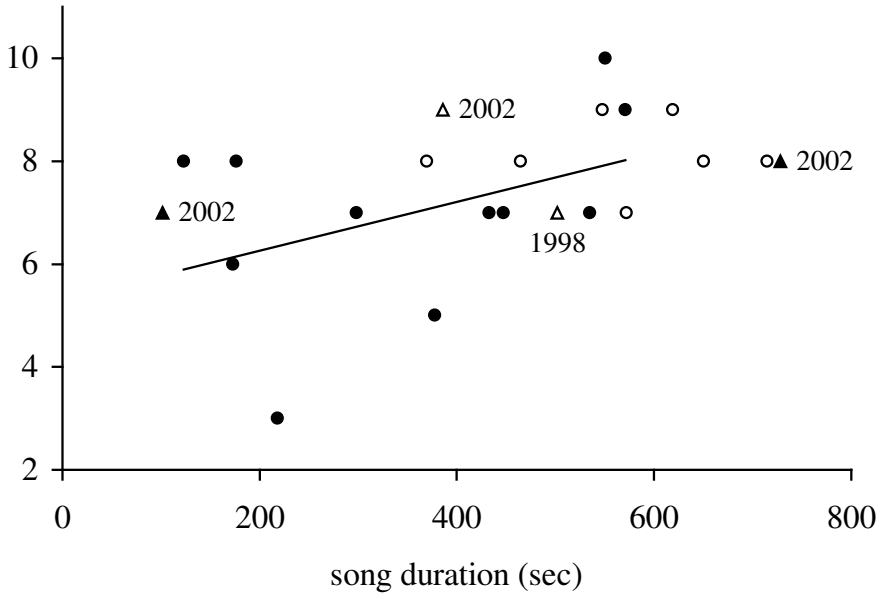
514 Figure 3



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